of beta-oxidation, PHA synthases with medium chain length substrate specificity, beta-ketothiolases, NADH or NADPH dependent reductases, PHA synthases with short chain length specificity, and PHA synthases that incorporate both short and medium chain length substrates.

Remarks

Claims 1, 2, 6-16, 18 and 20-28 are pending. Claims 1, 11, 12, 15, 16, 25 and 26 have been amended. Claims 3-5, 17 and 19 have been canceled. Support for the amendments made to claim 1 can be found, for example, at page 7, lines 23-28 (wherein the modified intein sequences are fused to the carboxy-terminus portion of each gene except the last gene to be expressed); and page 4, lines 10-12 (wherein the excised exteins are not ligated). Page 8, lines 4-7, for example, provides support for the above-identified amendments relating to DNA constructs encoding glycines or alanines that link the intein and extein amino acid sequences. All of the other above-identified amendments provide clarity to the claimed compositions and methods.

The present invention is directed toward constructs and methods directing the introduction of multiple genes into cells via a single transformation event. A splicing unit (intein) I engineered to promote excision of all non-essential components in a polyprotein and inhibit the ligation reactions between the excised exteins normally associated with protein splicing. The claimed constructs include additional genetic elements such as a 5' promoter and transcription termination sequences.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-2, 4, 6-16, 18, and 20-28 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

What is provided in the Examples is a molecular biology "cookbook" for the *in vivo* expression of two separate proteins from an intein containing multi-gene expression construct with only one promoter and one poly-adenylation signal. Example 1 clearly describes a construct harboring the 35S-C4PPDK light inducible plant promoter; an N-terminal extein sequence encoding beta-glucuronidase (GUS) fused at its C-terminus to the N-terminus of an intein sequence; an intein sequence from the *Pyrococcus* species GB-D polymerase in which serine 538 has been mutated to alanine or glycine; a 5'-terminal extein sequence encoding an enhanced green fluorescent protein fused at its 3'-terminus to the 5'-terminus of the intein sequence; and a polyadenylation signal. The construct is expressed in a plant protoplast system, and the proteins expressed from the construct are assayed *via* Western analysis (as described in the Example). The applicant respectfully submits that this is NOT a *prophetic* description, but rather a clearly defined example of a construct that harbors the essential elements required for proper expression and specific post-translational modifications (i.e. intein excision) of the proteins encoded therein. The protein products are easily detected using methods well known in the art.

The Examiner points to several references that illustrate the unpredictability of protein *splicing*. "Perler" (Cell, Vol. 92, 1-4; 1998) describes the mediation of protein cleavage and

ligation by inteins that are approximately 150 amino acids in length. Perler further describes a core endonuclease domain as a separate structural domain within the intein. Perler is generally directed to protein ***splicing***. Perler does not contemplate "splicing" broken down into its constituent activities (i.e. those separate activities required for "splicing"). For example, the inteins of Perler are defined as functioning properly if they splice, or ligate, ***ex***ternal pro***tein*** fragments (i.e. carry out the complete ligation process; see for example, page 1, 2nd column, 2nd paragraph, wherein "failure to ***splice*** in *E. coli* doesn't always indicate a defective intein...."; emphasis added). The applicant respectfully asserts that statements such as, "[P]rotein splicing is less efficient when an intein is expressed within a foreign protein" (see Perler, page 1, 2nd column, 3rd paragraph) and "[P]roximal foreign extein residues can potentially disturb the intein active-site by steric hinderance, etc. Anraku and coworkers have suggested that the N extein interacts with intein residues to align the splice sites" (see Perler, page 1, 2nd column, 3rd paragraph), are directed to the complete process of splicing, or ligating, two exteins. Based upon these observations, one of ordinary skill in the art would readily appreciate that a "failure to splice" could result in products identical to those produced using the applicant's claimed compositions and methods. If anything, the Perler reference, through its raising of red flags in the overall protein splicing process, may shed light onto the process by which inteins are excised and the resulting exteins remain free of subsequent ligation.

The Examiner tries to provide a nexus between the "Morassutti" reference (FEBS Letters 519; 2002) and the presently claimed methods and compositions. It appears that the Examiner fails to appreciate that the claimed methods and compositions are directed to the intracellular

production of separate proteins as described. Morassutti separates an intein fusion protein from leaf tissue homogenate *via* a G-50 Sephadex column and, subsequently, a chitin resin column. Once isolated, the intein fusion protein is induced to "self-cleave" *in vitro*. One of ordinary skill in the art would readily appreciate that induction of any chemical/enzymatic reaction *via* the addition of extraneous reagents is not uncommon, in view of those reactions that are forced into motion in an environment "outside" of their normal cellular location (such as those of Morassutti). In view of the foregoing, the applicant asserts that there would have been no need to address the *in vitro* reactions of Morassutti in the specification in order to provide an enabling disclosure for the claims as pending.

As stated above, the references cited to refute the applicant's assertion that the present disclosure is enabling, appear to be generally directed to the overall process of splicing, or trans-splicing ("Evans"; Evans *et al., The Journal of Biol. Chem.*, Vol. 275,13; 2000). Evans, per the Examiner's assertion, teaches that the number of C-terminal extein amino acid residues has an effect on protein splicing as does the temperature at which the organism is grown. Again, the present claims are not directed to splicing, *per se*. They are directed to the utilization of modified intein sequences to produce multiple gene products (i.e. the gene products are not spliced or ligated). Therefore, any failure of splicing reactions may result in the products generated as a result of expressing the claimed constructs, or the claimed methods.

In summary, none of the Examiner's cited references cast any doubt onto the level of predictability of the claimed invention asserted by the applicant; or the guidance provided in the specification as originally filed. The applicant respectfully reminds the Examiner that the inteins

used in the presently claimed methods and compositions are modified. Splicing (i.e. ligating two or more exteins) is not contemplated by the claims as pending. Again, the specification need only enable a person skilled in the art to make and use the claimed invention without undue experimentation. Page 18, lines 24-26, clearly provides the results of western analysis *via* SDS-PAGE of *in vivo* expression assays ("*in vivo* expression of two proteins from an intein containing multi-gene expression cassette"). The detection methods used in Example 1 incorporate western analysis *via* SDS-PAGE to separate and identify these individual proteins using proper immunological detection reagents. Page 21, lines 5-12, of the description, details the immunological method used to detect the two separate proteins produced from an intein containing multi-gene expression construct with only one promoter and one poly-adenylation signal in a plant protoplast. Therefore, the experimentation one of ordinary skill in the art would undertake to produce the claimed construct would not be considered undue.

Claims 1-2, 4, 6-16, 18 and 20-28 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Examiner asserts that the claimed "system" is designed using theory and that the components of the protein splicing process have been studied by previous researchers. The Examiner further asserts that "since an intein that can be used to separate proteins has not been

described by specific structural features or by specific function, the specification fails to provide an adequate written description....". The Examiner appears to be completely disregarding the section entitled "Inteins", on pages 7-9. One of ordinary skill in the art will readily interpret the section as clearly describing examples of known inteins, modified and unmodified, that harbor specific activities. Furthermore, as taught on these same pages, databases harboring "all known inteins" are readily available. These databases coupled with successful modification of inteins to produce a desired activity (i.e. induction of extein cleavage and removal of ligation activity, page 8, lines 1-5; removal of endonuclease activity, page 8, lines 7-9, lines 15-19; and engineering of inteins to contain the minimal number of amino acids in order to retain splicing function, page 8, lines 12-18) provide one of ordinary skill in the art with a well-defined "blueprint" for making and/or using the claimed invention commensurate with the scope of the claims. Furthermore, one of ordinary skill in the art will recognize that "equivalent" residues exist in intein splicing units "due to the conservation of amino acids at the C-terminal extein junction to the intein (see page 8, lines 5-7).

The Examiner asserts that there is a lack of description for the intein/extein splicing process (see page 4, lines 10-11, of the Office Action mailed on October 23, 2002), and then goes on to cite Chong *et al.* (under 35 U.S.C. § 102(b)) as providing evidence that the applicant's claims are anticipated by prior art. Chong clearly teaches that the protein splicing process was well known in the art at the time of filing the present application. In view of the specification, at page 2, lines 1-12, the Applicant respectfully submits that the mechanisms governing protein

splicing are conserved. Therefore, this particular assertion made by the Examiner simply does not make sense.

Rejection Under 35 U.S.C. § 102

Claims 1 and 15 were rejected under 35 U.S.C. § 102(b) as being anticipated by the *Journal of Biological Chemistry* 271:22159-22168 by Chong *et al.* ("Chong"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The claims as amended are now directed to DNA constructs, and methods, for expressing multiple genes in cells by making use of intein sequences that catalyze excision of exteins, wherein the excised exteins are not ligated. Chong fails to teach any construct or method that makes use of intein sequences that catalyze excision of exteins, *wherein the excised exteins are not ligated.* While Chong attempts to arrest the splicing of MYT (maltose binding protein-yeast VMA intein-*E. coli* thioredoxin) at specific stages, Figure 2 of Chong clearly fails to identify (and the specification fails to discuss), any proteins related to maltose binding protein and *E. coli* thioredoxin as separate entities.

Allowance of claims 1, 2, 6-16, 18, and 20-28 is respectfully solicited.

Respectfully submitted,

Todd S. Hofmeister
Reg. No. P-53,029

Date: January 23, 2003

HOLLAND & KNIGHT LLP
One Atlantic Center, Suite 2000
1201 West Peachtree Street
Atlanta, Georgia 30309-3400
(404) 817-8473
(404) 817-8588 (Fax)

Certificate of Mailing Under 37 C.F.R. § 1.8(a)

I hereby certify that this Amendment and Response to Office Action, and any documents referred to as attached or enclosed, is being deposited with the United States Postal Service on this date, January 23, 2003, with sufficient postage as first-class mail in an envelope addressed to the Commissioner for Patents, U.S. Patent and Trademark Office, Washington, DC 20231.

Todd S. Hofmeister

Date: January 23, 2003

Marked Up Version of Amended Claims
Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. (Amended) A DNA construct for expression of multiple gene products in a cell comprising:

(a) a single promoter at the 5' end of the construct,

(b) multiple genes, or exteins, encoding one or more proteins,

(c) [a first intein sequence fused to the portion of the gene encoding the carboxy-terminus of a first encoded protein] modified intein sequences, modified by fusing to the carboxy-terminus portion of each gene except the last gene to be expressed, and

[(d) a second intein sequence fused to the portion of the gene encoding the carboxy-terminus of a second encoded protein,

(e)](d) transcription termination sequences,

wherein at least [the first] one of the intein [sequence] sequences can catalyze excision of the exteins, and wherein the excised exteins are not ligated.

2. The construct of claim 1 for expression in a eucaryotic cell wherein the transcription termination sequences comprises a polyadenylation signal at the 3' end of the construct.

Please cancel claims 3-5.

6. The construct of claim 1 wherein the promoter is selected from the group consisting of inducible promoters, constitutive promoters and tissue specific promoters.

7. The construct of claim 1 wherein the genes encoding one or more proteins are preceded or followed by a sequence encoding a peptide that targets the gene expression product to a particular compartment within the cell in which the construct is expressed.

8. The construct of claim 1 wherein the proteins are different enzymes.

9. The construct of claim 1 wherein the proteins are the same proteins.

10. The construct of claim 1 wherein the inteins prevent the ligation reactions normally associated with protein splicing.

11. (Amended) The DNA construct of claim 10 wherein [one or more inteins comprise exteins and the first residue of the 3'-terminal extein is engineered to contain a glycine or alanine] the DNA construct encodes a glycine or alanine linking the intein and extein amino acid sequences.

12. (Amended) The construct of claim 4 wherein the proteins are selected from the group consisting of acyl CoA dehydrogenases[)], acyl CoA oxidases, catalases, alpha subunits of beta-oxidation, beta subunits of beta-oxidation, PHA synthases with medium chain length substrate specificity, beta-ketothiolases, NADH or NADPH dependent reductases, PHA synthases with short chain length specificity, and PHA synthases that incorporate both short and medium chain length substrates.

13. The construct of claim 4 wherein the proteins are selected from the group consisting of enzymes encoded by the phaG locus, medium chain length synthases, beta-

ketothiolases, NADH or NADPH dependent reductases, and PHA synthases that incorporate both short and medium chain length substrates.

14. The construct of claim 4 wherein the proteins are selected from the group consisting of herbicide resistance, insect resistance, and desirable plant crop traits.

15. (Amended) A method for expressing multiple genes in cells comprising transforming the cells with a DNA construct comprising:

a) a single promoter at the 5' end of the construct,

b) multiple genes, or exteins, encoding one or more proteins,

c) [a first intein sequence fused to the portion of the gene encoding the carboxy-terminus of a first encoded protein] modified intein sequences, modified by fusing to the carboxy-terminus portion of each gene except the last gene to be expressed, and

[(d) a second intein sequence fused to the portion of the gene encoding the carboxy-terminus of a second encoded protein,

(e)](d) transcription termination sequences,

wherein at least [the first] one of the intein [sequence] sequences can catalyze excision of the exteins, and wherein the excised exteins are not ligated.

16. (Amended) The method of claim 15 for expression in a [eucaryotic] eukaryotic cell wherein the transcription termination sequences comprises a polyadenylation signal at the 3' end of the construct.

Please cancel claim 17.

18. The method of claim 15 wherein the cell is a plant cell and the promoter is a promoter operable in a plant cell.

Please cancel claim 19.

20. The method of claim 15 wherein the promoter is selected from the group consisting of inducible promoters, constitutive promoters and tissue specific promoters.

21. The method of claim 15 wherein the genes encoding one or more proteins are preceded or followed by a sequence encoding a peptide that targets the gene expression product to a particular compartment within the cell in which the construct is expressed.

22. The method of claim 15 wherein the proteins are different enzymes.

23. The method of claim 15 wherein the proteins are the same proteins.

24. The method of claim 15 wherein the inteins prevent the ligation reactions normally associated with protein splicing.

25. (Amended) The method of claim 24 wherein [one or more inteins comprise exteins and the first residue of the 3'-terminal extein is engineered to contain a glycine or alanine] the DNA construct encodes a glycine or alanine linking the intein and extein amino acid sequences.

26. (Amended) The method of claim 18 for making polyhydroxyalkanoates in plants wherein the proteins are selected from the group consisting of acyl CoA dehydrogenases[)], acyl CoA oxidases, catalases, alpha subunits of beta-oxidation, beta subunits of beta-oxidation, PHA synthases with medium chain length substrate specificity, beta-

ketothiolases, NADH or NADPH dependent reductases, PHA synthases with short chain length specificity, and PHA synthases that incorporate both short and medium chain length substrates.

27. The method of claim 18 for making polyhydroxyalkanoates in plants wherein the proteins are selected from the group consisting of enzymes encoded by the phaG locus, medium chain length synthases, beta-ketothiolases, NADH or NADPH dependent reductases, and PHA synthases that incorporate both short and medium chain length substrates.

28. The construct of claim 18 wherein the proteins are selected from the group consisting of herbicide resistance, insect resistance, and desirable plant crop traits.